Applicants:

R. Timmis, et al.

Attorney Docket No.: WEYE116514/22822A

Application No.:

09/700,037

Group Art Unit: 1651

Filed: July 2, 2001

Examiner: L.B. Lankford Jr.

Title: METHODS FOR CLASSIFICATION OF SOMATIC EMBRYOS

STATEMENT BY JAMES A. GROB

Federal Way, Washington 98063

October 13, 2004

TO THE COMMISSIONER FOR PATENTS:

- 1. I, James A. Grob, resident of 10404 Springwood Drive East, Bonnie Lake, WA 98390, and employee of Weyerhaeuser Company, have been named as an inventor for the invention claimed in the above-referenced US patent application.
- 2. I hereby confirm that I am no longer an inventor for the invention claimed in the subject matter, due to changes in the claims in the course of prosecution. The inclusion of my name as inventor is no longer appropriate.
- 3. There was no deceptive intent in my inclusion as an inventor in this case, nor is there deceptive intent in my request to be deleted as an inventor in this case.
- 4. Accordingly, I request that I be removed as an inventor from this application.

James A. Grob Date

Applicants:

R. Timmis, et al.

Attorney Docket No.: WEYE116514/22822A

Application No.:

09/700,037

Group Art Unit: 1651

Filed: July 2, 2001

Examiner: L.B. Lankford Jr.

Title: METHODS FOR CLASSIFICATION OF SOMATIC EMBRYOS

STATEMENT BY WILLIAM C. CARLSON

Federal Way, Washington 98063

October 13, 2004

TO THE COMMISSIONER FOR PATENTS:

- I, William C. Carlson, resident of 7211 48th Way NW, Olympia, WA 98502-9523, and employee of Weyerhaeuser Company, have been named as an inventor for the invention claimed in the above-referenced US patent application.
- I hereby confirm that I am no longer an inventor for the invention claimed in the subject matter, due to changes in the claims in the course of prosecution. The inclusion of my name as inventor is no longer appropriate.
- 3. There was no deceptive intent in my inclusion as an inventor in this case, nor is there deceptive intent in my request to be deleted as an inventor in this case.
- 4. Accordingly, I request that I be removed as an inventor from this application.

William C. Carlson

10/12/04 Date

Applicants:

R. Timmis, et al.

Attorney Docket No.: WEYE116514/22822A

Application No.: 09/700,037

Group Art Unit: 1651

Filed:

July 2, 2001

Examiner: L.B. Lankford Jr.

Title:

METHODS FOR CLASSIFICATION OF SOMATIC EMBRYOS

DECLARATION UNDER 37 C.F.R. § 1.132

Seattle, Washington 98101

TO THE COMMISSIONER FOR PATENTS:

We, Roger Timmis, Mitchell R. Toland, and Timnit Ghermay, do hereby declare that:

- 1. We are the co-inventors of the invention claimed in the above-identified patent application.
- 2. We had been working towards devising methods for automatically classifying somatic embryos according to their putative quantifiable characteristics, such as their tendency to successfully germinate, to tolerate adverse weathers, diseases, etc. Our effort resulted in the methods as claimed in the above-identified patent application.
- 3. Specifically, we have discovered that, by applying one or more classification algorithms to the spectral raw data collected from plant embryos of known quantifiable characteristics, we could develop a classification model that can then be used to classify plant embryos of unknown quantifiable characteristics, according to their putative quantifiable characteristics.
- 4. According to one embodiment, the method of the present invention involves generally three steps. First, spectral data are obtained from reference plant embryos having known quantifiable characteristics. In a simple case, the reference plant embryos are divided into two groups: those of relatively high quality and those of relatively low quality based, for example, on a follow-up study.

Second, one or more classification algorithms are applied to the spectral data sets (2) sets in this example), each being associated with certain known quantifiable characteristics, to develop a classification model for classifying plant embryos according to their presumed quantifiable characteristics. Various classification algorithms are available to develop a

classification model. In one example described in the specification of the present application, at page 37, line 13 through page 38, line 10, an NIR (near infrared) spectroscopic set-up was used to collect spectral data from a set of reference plant embryos of known quality, and the collected spectral data were subjected to principal components analysis to develop a classification model. Principal component analysis may be applied to the collected spectral data using a software package "The Unscrambler" available from Camo ASA, and the results of the analysis in one example are shown in FIGURE 2A. In this example, reference plant embryos were divided into four groups: Douglas-fir zygotic embryos of three different developmental stages (mature dry zygotics - noted as black circles in FIGURE 2A; "August 14" immature zygotics – white triangles; and "July 23" immature zygotics – black squares) and somatic embryos from Genotype 1 ("+" symbols). FIGURE 2A shows that these four populations of varying embryo quality can be separated into four clearly distinct groups when plotted with respect to the first three principal components obtained from the principal component analysis. In other words, the spectral data collected from the reference plant embryos and analyzed using principal component analysis are clearly "correlated" to the four populations of varying quality. Thus, these results are used to form a classification model to classify a plant embryo of unknown embryo quality.

Third, spectral data is obtained from a plant embryo of unknown quality, and the developed classification model is applied to the obtained spectral data to classify the plant embryo according to its putative quantifiable characteristics.

5. As the above example illustrates, we were able to establish a clear correlation between spectral raw data collected from plant embryos and the plant embryos' quantifiable characteristics, by applying various classification algorithms to the spectral raw data. We have demonstrated, in various examples shown in the specification, that based on such correlation, a classification model can be developed and applied to embryos of unknown quality to classify them according to their putative quantifiable characteristics. We have successfully implemented some exemplary classification models, as shown in the specification.

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Date	Roger Timmis
Date	Mitchell Toland
Date	Timnit Ghermay

Date	Roger Timmis
12 Oct 2004 Date	Mitchell Toland
Date	Timnit Ghermay

	6.	We acknowledge that willful false statements, and the like, are punishable by
fine or	impris	sonment, or both (18 U.S.C. § 1001), and may jeopardize the validity of the
applica	tion or	any patent issuing therefrom. All statements made of our own knowledge are
true and	d all sta	atements made on information and belief are believed to be true.

Date	Roger Timmis
Date	Mitchell Toland
Nicvember 1, 2004	Timnit Ghermay

Applicants:

R. Timmis, et al.

Attorney Docket No.: WEYE121906/22822B

Application No.: 09/700,037

Group Art Unit: 1651

Filed:

July 2, 2001

Examiner: L.B. Lankford, Jr.

Title:

METHODS FOR CLASSIFICATION OF SOMATIC EMBRYOS

DECLARATION UNDER 37 C.F.R. § 1.132

Seattle, Washington 98101

TO THE COMMISSIONER FOR PATENTS:

We, Roger Timmis, Mitchell R. Toland, Timnit Ghermay, William C. Carlson and James A. Grob, do hereby declare that:

- 1. We are the co-inventors of the invention claimed in the above-identified patent application.
- 2. We had been working towards devising methods for automatically classifying somatic embryos according to their putative quantifiable characteristics, such as their tendency to successfully germinate, to tolerate adverse weathers, diseases, etc. Our effort resulted in the methods as claimed in the above-identified patent application.
- 3. Specifically, we have discovered that, by applying one or more classification algorithms to raw image data, and specifically more than embryo perimeter data from raw image data collected from plant embryos of known quantifiable characteristics, we could develop a classification model that can then be used to classify plant embryos of unknown quantifiable characteristics, according to their putative quantifiable characteristics.
- 4. According to one embodiment, the method of the present invention involves generally three steps. First, image data are obtained from reference plant embryos having known quantifiable characteristics. In a simple case, the reference plant embryos are divided into two

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sets: those of relatively high quality and those of relatively low quality based, for example, on a

follow-up study.

Second, one or more classification algorithms are applied to more than embryo perimeter

data from the image data sets (2 sets in this example), each being associated with certain known

quantifiable characteristics, to develop a classification model for classifying plant embryos

according to their presumed quantifiable characteristics. Various classification algorithms are

available to develop a classification model. In one example described in the specification of the

present application, at page 21, line 3 through line 16, a digital imaging set up for collecting

digital images of a set of reference plant embryos of known quality is described. This set up was

used in Example 4 as described at page 24, line 9. Example 4 at page 24, line 20 through line 25,

describes that "when metrics image data was captured and added to the preprocessed image data

following the methods in Example 1, the accuracy of embryo classification into germinating and

nongerminating embryos was increased." Example 4, beginning at page 26, line 5, goes on to

describe that a classification method based on a Lorenz curve classification algorithm was

carried out on Douglas fir genotypes 6 and 7. This classification method based on a Lorenz

curve classification algorithm is described in more detail at page 27, line 17 through page 32,

line 1 of Example 4, and page 18, line 13 through line 34 of the specification. Page 18, line 12

through line 34, describes how metrics are correlated to the classes of embryos of differing

quality using the Lorenz curve classification algorithm.

Tables 4 and 5 list the morphology classification results and the germination

classification results from a method that uses a classification algorithm that uses more than

embryo perimeter data from the acquired image data. In Example 4, the nonperimeter image

data is correlated with plant embryos classified based on morphological similarity to normal

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zygotic embryos or germination classes. In Example 4, this correlation was used to develop an

embryo classification model.

The second column of Tables 4 and 5 list examples of metrics other than perimeter data

used by the Lorenz curve classification algorithm. These metrics include skewness coefficient

 (β_1) of all intensity values from the embryo end view; standard deviation of all green pixel

values from the end view; and kurtosis coefficient (β_2) of all green pixel values from the embryo

side view.

Once the classification model is developed, image data is obtained of plant embryos of

unknown embryo quality. The developed classification model that uses more than embryo

perimeter data is applied to the raw digital image data from the plant embryos of unknown

quality, and the quality of the plant embryos of unknown quality is classified.

5. As the above example illustrates, we were able to establish a clear correlation

between raw image data collected from plant embryos and the plant embryos' quantifiable

characteristics, by applying various classification algorithms that used more than embryo

perimeter data from the raw image data. We have demonstrated, in various examples shown in

the specification, that based on such correlation, a classification model can be developed and

applied to embryos of unknown quality to classify them according to their putative quantifiable

characteristics. We have successfully implemented some exemplary classification models, as

shown in the specification.

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Date		To an include the second	Timnit Ghermay
Date			William C. Carlson
——			Iames A Grob

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Date \	Roger Timmis
Date	Mitchell Toland
Date	Timnit Ghermay
Date	William C. Carlson
Date	James A. Grob

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Date	Roger Timmis
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Feb 18, 2005 Date	James A. Grob

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2/13/05 Date	Timnit Ghermay
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